

Pollen grain expression of osmotic adjustment as a screening method on drought tolerance in several wine and table grape genotypes (*Vitis vinifera* L.)

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Abstract

Osmotic adjustment is one of the important mechanisms to adapt to drought and it is the only one which is activated under any level of water stress in the plant cells. Grapevine pollen grains response was tested to osmotic stress in fourteen genotypes, initiated by immersion in 55% or 65% polyethylene glycol solutions without and with addition of potassium chloride, to estimate the expressions of osmotic adjustment. The pollen grain test found differences both in the measurements of projected area cytoplasm and expressions of osmotic adjustment present in the cells. Italian Riesling increased pollen grains cytoplasm in PEG solutions with added KCl much more than other genotypes and had the high values for both expressions of induced and overall osmotic adjustment. The results obtained for expression of induced osmotic adjustment underlined the high K⁺ accumulation capacity of 'Italian Riesling', 'Burgund mare' 86 Șt., 'Muscat d'Adda' 22 Șt., 'Muscat Ottonel' 16 Șt., 'Pinot gris' 14 Șt. and 'Argessis'. The lack of correlation between expressions of induced and intrinsic osmotic adjustment indicated that induced osmotic adjustment expressed by K⁺ might use different mechanisms that are activated at the time of water stress with different levels of solute accumulation. Because the accumulation of K⁺ in the cells is important in all developmental stages and, in grape yield and quality, pollen responses to induced osmotic adjustment expressed by K⁺ could be used as a screening method, for establishing the level of drought sensitivity in the grape varieties under water stress.

Keywords: cytoplasm; grapevine clones; osmosis; plasmolysis; water deficit

Abbreviations: ABA-abscisic acid; KCl-potassium chloride; Mg²⁺-magnesium ion; OA-osmotic adjustment; PEG(K⁺)-polyethylene glycol with addition of KCl

Introduction

The frequency of extreme drought is predicted to increase, influencing negatively the yield and quality of grapes. Drought may lead to massive leaf shedding with a consequent source-sink imbalance and incomplete berry maturation due to insufficient available carbohydrates (Chaves *et al.*, 2010). For understanding

physiological and molecular bases of plant responses to mild and moderate water deficits, Blum (2009) has been recommended that the research must be conducted between vegetative and reproductive development, aiming to find the best solutions for improving crop water use and controlling fruit quality under drought conditions (Chaves *et al.*, 2007, 2010).

Osmotic adjustment capacity, which allows maintaining the plant cell turgescence by accumulation of organic or inorganic osmolytes as response to water stress, is one of the important characteristics to adapt to water deficit (Morgan, 1983, 1999). Many crop plants, and also woody shrubs and trees, respond to drought conditions by intracellular net accumulation of solutes, as has been documented in sorghum (Jones and Turner, 1980), wheat (Morgan, 1980; Morgan *et al.*, 1986; Kikuta and Richter, 1988), corn (Sobrado, 1990) and several trees species (Parker and Pallardy, 1985; Sobrado, 1986; Tschaplinski and Blanke, 1989; Rodrigues *et al.*, 1993). As osmoticum adjustment is a cellular mechanism, it is expressed in all plant cells, including pollen grains and this offers a convenient way to characterize germplasm for this trait (Morgan, 1999; Moud and Yamagishi, 2005).

Patil and Ravikumar (2011) have been differentiated two responses of pollen projected area to osmotic stress, in their work on sorghum. The response induced by stress solutions (PEG) without addition of inorganic osmolyte was considered as expression of “intrinsic” osmotic adjustment, while the response to external supply of inorganic osmolyte to the stress solutions was described as expression of “induced” osmotic adjustment. Researches on wheat plants identified large differences among cultivars, given by these two types of pollen grain responses to osmotic stress (Morgan *et al.*, 1986; Bănică *et al.*, 2008; David, 2009; David, 2012). The K⁺, as an inorganic osmolyte, has an important role in pollen hydration and pollen tube growth under water stress in grapevine, being controlled by inward K⁺ channel activity (Mouline *et al.*, 2002; Zhao *et al.*, 2013; Cuéllar *et al.*, 2013; Rogiers *et al.*, 2017).

Following the pollen grain test performed as a screening method on drought sensitivity in other crop plants, this paper presents the results obtained for estimation of induced and intrinsic OA expressions present in the cells, using quantitative measurements of projected pollen grain cytoplasm area, in a diverse grapevine set, aiming to characterize wine and table grape varieties for drought tolerance response.

Materials and Methods

Plant material

A set of fourteen grapevine genotypes were studied for their pollen grain expressed osmotic adjustment, using the test of pollen grain developed by Morgan (1999) on wheat plants.

The varieties were characterized by Glăman *et al.* (2018) as:

1. Drought tolerant varieties: ‘Italian Riesling’, ‘Victoria’, ‘Argessis’, ‘Burgund mare’ 86 Şt.; ‘Fetească albă’ 97 Şt.; ‘Muscat d’Adda’ 22 Şt.; ‘Muscat Ottonel’ 16 Şt.; ‘Pinot gris’ 14 Şt.; ‘Pinot noir’ 3 Şt.; ‘Şarbă’ 2 Şt.; ‘Sauvignon petit’ 111 Şt. and ‘Chardonnay’ 15 Şt.
2. Drought sensitive cultivar: ‘Tămâioasă Românească’.
3. No information about drought sensitivity: ‘Ştefăneşti’.

Greenhouse conditions

Grapevine genotypes (*Vitis vinifera* L.) of 10 years old were planted in pots (20 dm³) containing a soil:peat:sand mixture (3:1:0.5). Inside the greenhouse, the maximum and means of temperature were 27 °C and 18 °C, respectively, at the time when flowers at anthesis were collected. The humidity was between 30% and 80%, respectively. The grapevine plants received drip irrigation to induce moderate water stress.

Grapevine pollen test

Grapevine pollen grains are similar with those of wheat plants (Oprea and Indreăş, 2000). Therefore, the procedure of wheat pollen test developed by Morgan (1999) was used on grapevine pollen grains. The pollen grains of matured anthers were soaked in polyethylene glycol (PEG 10,000) solutions of 55% and 65%, over microscope slides, with and without addition of 10 mM KCl for each concentration of PEG solutions. After a little agitation to release the pollen grains, the anther sections were removed and then, the solutions covered with a cover slip. The slides were incubated at 20 °C for 1 day. Microscopic observations were made using a magnification of 20X. A stressing concentration of PEG induced shrinkage of the pollen grains cytoplasm, which assumed a more conical shape, often with concavities.

The following parameters were recorded (Patil and Ravikumar, 2011):

A-Initial cytoplasm area (Control) induced in PEG solution of 30%.

B-The projected cytoplasm area of pollen grains, after 24 hours of osmotic stress induced by 55% and 65% PEG solutions.

C-The projected cytoplasm area of pollen grains, after 24 hours of incubation in 55% and 65% PEG solutions with addition of 10 mM KCl to solutions.

Measurements of projected pollen grains cytoplasm were made using the Quickphoto2.3 tools designed by Olympus Imaging Software.

Estimation of osmotic adjustment expressions induced in grapevine pollen grains

Based on the theory proposed by Patil and Ravikumar (2011), estimation of osmotic adjustment expressions induced in grapevine pollen grains was determined using:

1. The ratios (B/A) of projected pollen cytoplasm area induced under stress (mean of both 55% and 65% PEG without KCl) on the non-stressed initial cytoplasm area, as a measure of intrinsic OA.

2. The ratios (C/B) of projected pollen cytoplasm area induced under stress with external osmolyte supply (mean of 55% and 65% PEG with addition of KCl) on the projected pollen cytoplasm area induced under stress (mean of 55% and 65% PEG without KCl), as a measure of induced OA.

3. The ratios (C/A) of projected pollen cytoplasm induced under stress with external osmolyte supply (mean of 55% and 65% PEG with addition of KCl) on non-stressed initial cytoplasm area, as a measure of overall OA.

Ratio coefficients expressed in means obtained for estimation of osmotic adjustment response at different levels of osmotic stresses induced in the pollen grains which had values ≥ 1 , indicated in our screening the level of drought sensitivity in grapevine genotypes for each OA expression.

Statistical analysis

The statistical interpretation of the results was conducted using analysis of variance (Fischer-Snedecor test). Analysis of variance was performed in Excel for each treatment and trait in three repetitions given by measurements of projected pollen grain cytoplasm area made on the images focused in three central locations of the slide observed at magnification of 20X. The significance of results was compared through LSD test (Steel *et al.*, 1997), corresponded to a significant limit of 5%. The results of LSD test were expressed both in absolute and relative values. Analysis of the relationships among traits given by expressions of osmotic adjustment were examined through calculation of correlation coefficients (R).

Results

Because when pollen grains are immersed in water or 15% PEG, they decreased and increased in size and/or burst, the initial cytoplasm area was considered the area measured after 24 hours of immersion in a non-stressing solution of 30% PEG (Figure 1).

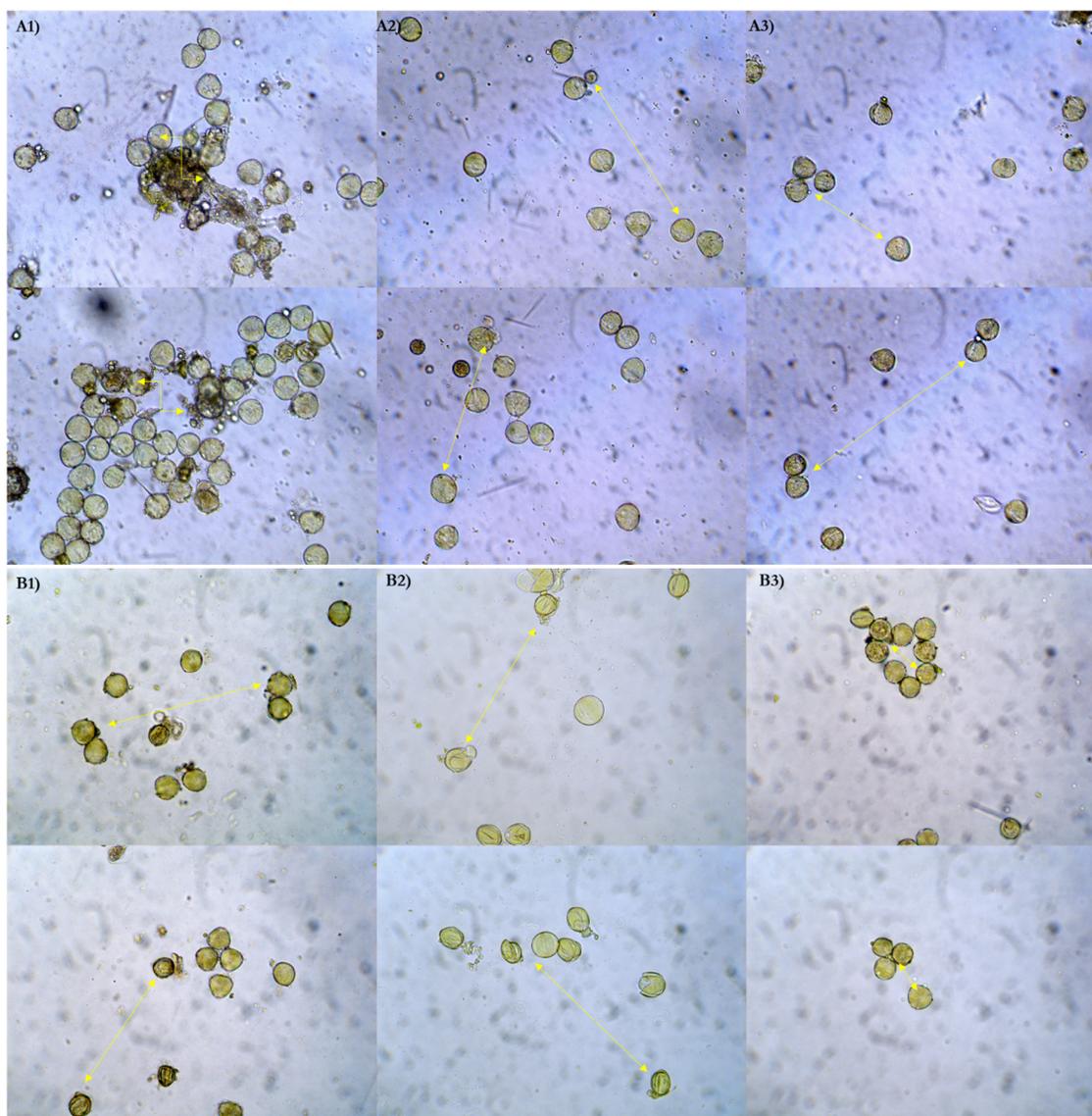


Figure 1. Pollen grain expressions of two grapevine cultivars in different control treatments given by image repetitions focused on Olympus microscope at magnification of 20X (A1.) 'Argessis' pollen grains exposed to distillate water, modulated by a decreasing, and increasing in cytoplasm area or by broken cells (arrows); (A2.) 'Argessis' pollen grains exposed to 15% PEG: the same pollen grains expression as in images A1. (arrows); (A3.) 'Argessis' pollen grains exposed to 30% PEG: homogeneous expression of pollen grains cytoplasm (arrows); (B1.) Pollen grains of 'Ștefănești' exposed to distillate water, modulated by a decreasing, and increasing in cytoplasm area or by broken cells (arrows); (B2.) Pollen grains of 'Ștefănești' exposed to 15% PEG modulated by broken cells (arrows); (B3.) Pollen grains of 'Ștefănești' exposed to 30% PEG: homogeneous expression of pollen grains cytoplasm (arrows).

Differences between genotypes regarding the projected area of pollen grains cytoplasm in non-stressing and stressing PEG solution

Large differences were found in the measurements expressed in means of the projected cytoplasm area of pollen grains, subjected to various levels of osmotic stress in PEG solutions, among the studied grapevine genotypes (Table 1).

The means of projected pollen cytoplasm area for all 14 grapevine genotypes expressed in 55% and 65% PEG solutions were smaller than the mean of pollen cytoplasm area expressed as control (30% PEG) by 29.96%

and 34.87%, respectively. Adding KCl to the PEG solutions, the means of projected pollen cytoplasm area were smaller than the mean of control by 22.16% and 28.57%, respectively (Table 2).

Grapevine genotypes had different responses to the applied osmotic stress treatments. Each pollen grain cell was measured on the slide. As in wheat pollen grain test, at the microscopic level, the lack of cell uniformity given by grape pollen grains conducted to large standard deviations calculated for each genotype (Table 2).

The pollen grain responses to different osmotic stresses were different among grapevine genotypes (Figure 2). Grapevine projected area of pollen cytoplasm decreased after immersion in PEG solutions by more than 21.3% in 'Şarbă' 2 Şt. or 46.8% in 'Tămăioasă Românească' (Figure 2 (A*.; C*.; D*.) and greatly increased with 27.3% in 'Italian Riesling' or 23.5% in 'Victoria' (Figure 2 (E*.)), after addition of KCl in the PEG solutions. The projected area of pollen cytoplasm barely changed by less than 2.7% in 'Sauvignon petit' 111 Şt., 2.5% in 'Fetească albă' 97 Şt. or (-) 3.3% in 'Chardonnay' 15 Şt. (Figure 2 (E*.)).

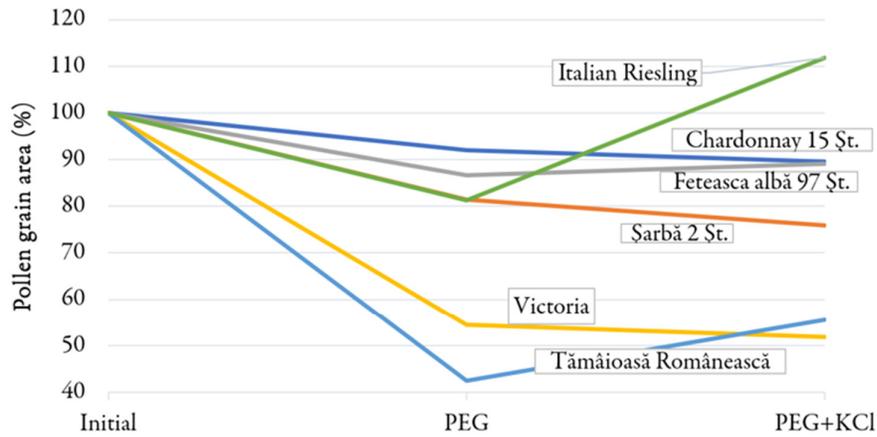
Table 1. ANOVA of measurements expressed in means of projected area of pollen grains cytoplasm

ANOVA of:				
Projected pollen cytoplasm area in 55% PEG	DF	MS	Fischer Factor	Pr(>F)
Variables as varieties	13	120,954.05	32.64**	2.09
Estimation of general errors determined by all experimental differences	28	3,706.19		
Projected pollen cytoplasm area in 55% PEG(K ⁺)	DF	MS	Fischer Factor	Pr(>F)
Variables as varieties	13	164,412.97	65.05***	2.09
Estimation of general errors determined by all experimental differences	28	2,527.50		
Projected pollen cytoplasm area in 65% PEG	DF	MS	Fischer Factor	Pr(>F)
Variables as varieties	13	257,259.54	65.21***	2.09
Estimation of general errors determined by all experimental differences	28	3,945.06		
Projected pollen cytoplasm area in 65% PEG(K ⁺)	DF	MS	Fischer Factor	Pr(>F)
Variables as varieties	13	295,520.71	41.01**	2.09
Estimation of general errors determined by all experimental differences	28	7,205.31		

F values (bold) were significant for P < 0.05; where DF means degrees of freedom; MS means mean square; Fischer factor means practical F; Pr(>F) means theoretical F

Table 2. Means ± standard deviations of projected pollen cytoplasm area (µm²) expressed in control (30% PEG) and stressing solutions without and with addition of KCl; LSD values were corresponded to a significant limit of 5% and were expressed both, in absolute and relative values

Genotype	Means of projected pollen cytoplasm area (µm ² ± standard deviation) expressed after exposure to solutions of:				
	30% PEG	55% PEG	65% PEG	55% PEG(K ⁺)	65% PEG(K ⁺)
Chardonnay 15 Şt.	1,039.87 ± 633.1	966.47 ± 402.8	946.17 ± 213.6	866.27 ± 564.4	996.07 ± 200.7
Şarbă 2 Şt.	1,461.53 ± 579.9	1,341.10 ± 252.1	1,040.10 ± 358.2	1,196.20 ± 247.3	1,022.18 ± 302.7
Feteasca albă 97 Şt.	1,365.73 ± 408.2	1,234.17 ± 151.2	1,131.57 ± 205.4	1,314.40 ± 233.3	1,118.87 ± 232.9
Pinot noir 3 Şt.	1,242.63 ± 504.1	1,055.37 ± 225.7	789.33 ± 172.4	1,113.70 ± 135.3	825.27 ± 136.3
Italian Riesling	970.73 ± 346.9	812.63 ± 154.73	765.43 ± 160.5	1,070.47 ± 359.0	1,101.20 ± 216.7
Muscat d'Adda 22 Şt.	1,514.10 ± 328.6	1,101.23 ± 236.4	1,158.03 ± 319.0	1,379.30 ± 212.6	1,430.63 ± 262.3
Ştefăneşti	1,538.07 ± 316.8	1,090.30 ± 157.2	1,221.23 ± 225.9	1,055.23 ± 239.4	1,083.20 ± 306.7
Argessis	1,597.67 ± 216.8	1,107.13 ± 304.2	1,430.73 ± 271.2	1,259.77 ± 304.4	1,631.70 ± 187.0
Burgund mare 86 Şt.	1,267.02 ± 448.6	865.60 ± 349.9	1,044.2 ± 261.8	1,520.87 ± 288.8	950.07 ± 344.2
Pinot gris 14 Şt.	1,357.97 ± 396.6	891.3 ± 262.17	417.27 ± 131.1	1,066.93 ± 136.6	422.33 ± 117.7
Sauvignon petit 111 Şt.	2,170.47 ± 534.2	1,370.93 ± 398.3	1,296.23 ± 388.5	1,354.97 ± 282.8	1,388.17 ± 241.9
Muscat Ottonel 16 Şt.	1,595.73 ± 284.5	923.03 ± 299.3	519.95 ± 169.1	1,015.33 ± 322.2	743.03 ± 220.1
Tămăioasă Ro.	1,262.33 ± 457.7	671.37 ± 201.17	703.53 ± 188.8	585.57 ± 276.7	724.20 ± 156.3
Victoria	2,077.03 ± 235.8	899.10 ± 320.97	862.43 ± 237.7	1,127.07 ± 253.1	1,178.40 ± 419.1
Average	1,461.49	1,023.55	951.87	1,137.57	1,043.95
LSD 5%-abs. values	73.22	34.98	36.09	28.89	48.78
LSD 5%-rel. values	5.01	3.42	3.79	2.54	4.67



(A)

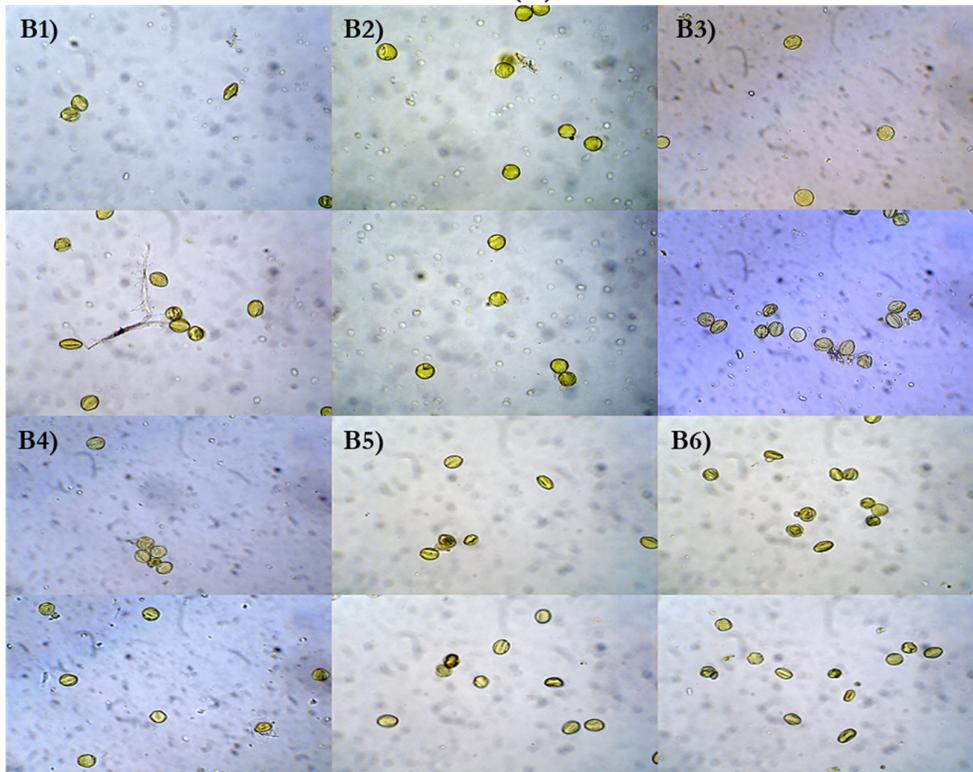
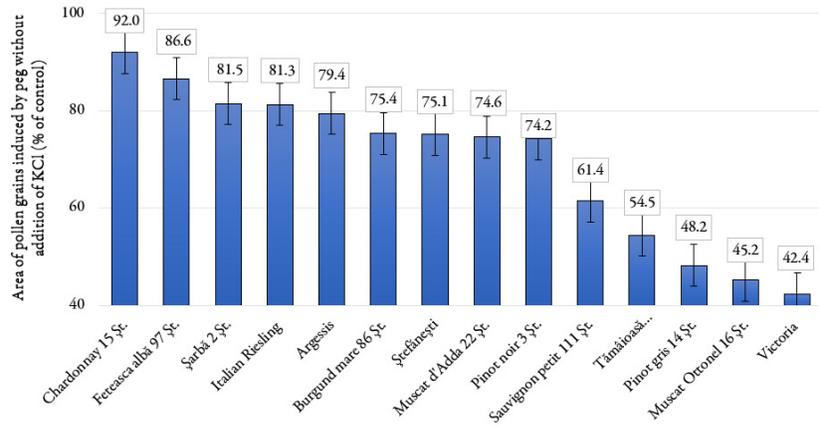
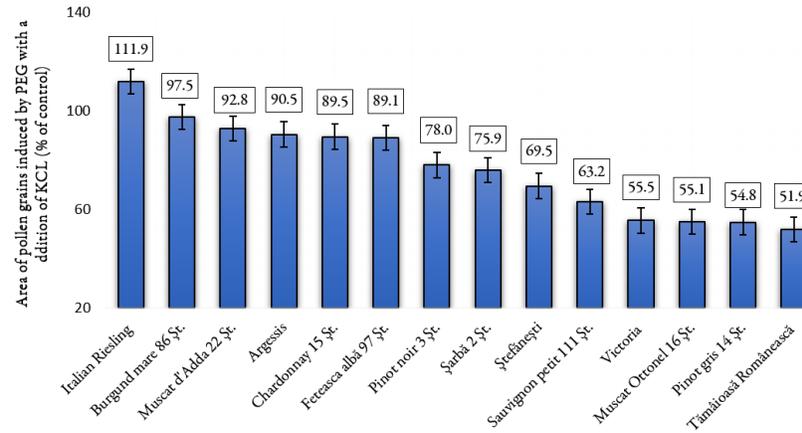


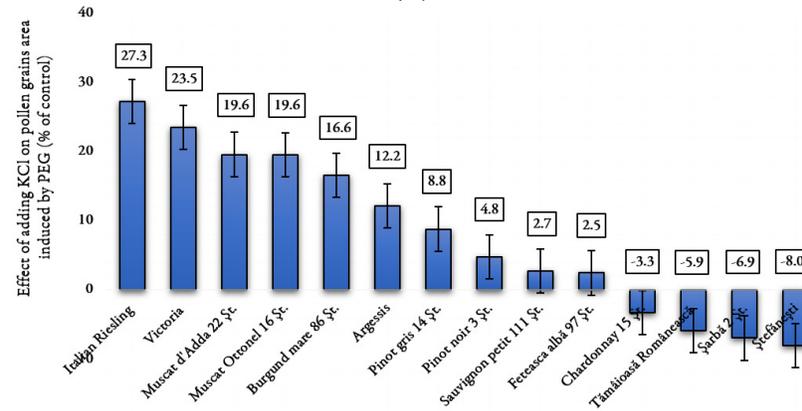
Figure 2. I. (A.) Pollen grain responses to different osmotic stresses applied in some representative grapevine genotypes; **(B.)** Pollen cytoplasm responses to different osmotic stresses with or without KCl for three representative grapevine genotypes as contrasting expressions substantiated by intrinsic and induced osmotic adjustment given by image repetitions focused on Olympus microscope at magnification of 20X: **B1)** 'Italian Riesling' pollen grains exposed to 65% PEG – cell shape changes because of the decreasing in initial projected cytoplasm; **B2)** 'Italian Riesling' pollen grains exposed to 65% PEG(K⁺) – reinitialization and increasing of initial projected cytoplasm in pollen grain cells; **B3)** 'Chardonnay' 15 Şt. pollen grains exposed to 65% PEG – partial reinitialization of initial projected cytoplasm in pollen grain cells; **B4)** 'Chardonnay' 15 Şt. pollen grains exposed to 65% PEG(K⁺) – cell shape changes because of the decreasing in initial projected cytoplasm; **B5)** 'Tămâioasă Românească' pollen grains exposed to 55% PEG – cell shape changes because of the decreasing in initial projected cytoplasm; **B6)** 'Tămâioasă Românească' pollen grains exposed to 55% PEG(K⁺) – cell shape changes because of the decreasing in initial projected cytoplasm



(C)



(D)



(E)

Figure 2. II. (C*.; D*.; E*.) Distribution of means \pm standard deviations ($n = 5$) for projected pollen grains cytoplasm expressed in % of control, at different levels of osmotic stress. * means that the figure took in consideration effect of PEG solutions with or without addition of KCl as a mean of two concentrations given by 55% and 65%

Differences between genotypes regarding expressions of osmotic adjustment

Large differences were found in the means of ratio coefficients calculated for expressions of intrinsic, overall and induced osmotic adjustment, among the studied grapevine genotypes (Table 3).

Table 3. ANOVA of ratio coefficients expressed in means which estimate different types of osmotic adjustment expressions induced in pollen grains; F values (bold) were significant for $P < 0.05$; where DF means degrees of freedom; MS means mean square; Fischer factor means practical F; $Pr(>F)$ means theoretical F

ANOVA of:				
Intrinsic OA expressed in 55% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.070	8.39*	2.09
Estimation of general errors determined by all experimental differences	28	0.008		
Intrinsic OA expressed in 65% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.125	11.36*	2.09
Estimation of general errors determined by all experimental differences	28	0.011		
Overall OA expressed in 55% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.130	14.14*	2.09
Estimation of general errors determined by all experimental differences	28	0.009		
Overall OA expressed in 65% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.154	16.56*	2.09
Estimation of general errors determined by all experimental differences	28	0.009		
Induced OA expressed in 55% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.161	23.59**	2.09
Estimation of general errors determined by all experimental differences	28	0.007		
Induced OA expressed in 65% PEG	DF	MS	Fischer Factor	$Pr(>F)$
Variables as varieties	13	0.099	7.40*	2.09
Estimation of general errors determined by all experimental differences	28	0.013		

Two close responses for expression of intrinsic osmotic adjustment (B/A) were found among grapevine genotypes. The limits of variations were from 0.424 ± 0.019 in 'Victoria' to 0.885 ± 0.152 in 'Fetească albă' 97 Șt. and 0.932 ± 0.157 in 'Chardonnay' 15 Șt. (Table 4).

The expression of induced osmotic adjustment (C/B) varied among grapevine genotypes from 0.926 ± 0.075 in 'Ștefănești' and 0.939 ± 0.058 in 'Șarbă' 2 Șt. to 1.384 ± 0.138 in 'Italian Riesling', 1.337 ± 0.075 in 'Burgund mare' 86 Șt. and 1.311 ± 0.072 in 'Victoria' (Table 4).

The limits of variation for overall osmotic adjustment expression were from 0.527 ± 0.224 in 'Tămâioasă Românească' to 1.123 ± 0.275 in 'Italian Riesling' (Table 4).

Relationships between expressions of osmotic adjustment in the grapevine varieties set

The genotype responses to addition of K^+ to PEG solutions (C/B) were not correlated with expression of intrinsic OA (B/A) (Figure 3). The negative relationship between these two indicators suggested that these two expressions of osmotic adjustment given by means of ratio coefficients (Table 4) analyzed for all fourteen grapevine genotypes represent mechanisms which action independently under drought conditions (Figure 3).

Table 4. Means \pm standard deviations of ratio coefficients which estimate different types of osmotic adjustment expressions induced in pollen grains; LSD values were corresponded to a significant limit of 5% and were expressed both, in absolute and relative values

Genotype	Expression of induced OA	Expression of overall OA	Expression of intrinsic OA
Italian Riesling	1.384 \pm 0.138	1.123 \pm 0.275	0.818 \pm 0.090
Burgund mare 86 Şt.	1.337 \pm 0.075	0.981 \pm 0.254	0.759 \pm 0.073
Victoria	1.311 \pm 0.072	0.555 \pm 0.161	0.424 \pm 0.019
Muscat Ottonel 16 Şt.	1.269 \pm 0.101	0.551 \pm 0.138	0.452 \pm 0.039
Muscat d'Adda 22 Şt.	1.244 \pm 0.023	0.935 \pm 0.259	0.751 \pm 0.057
Argessis	1.143 \pm 0.086	0.906 \pm 0.184	0.795 \pm 0.038
Pinot gris 14 Şt.	1.113 \pm 0.091	0.560 \pm 0.266	0.492 \pm 0.090
Pinot noir 3 Şt.	1.053 \pm 0.110	0.782 \pm 0.233	0.743 \pm 0.038
Feteasca albă 97 Şt.	1.031 \pm 0.100	0.903 \pm 0.291	0.885 \pm 0.152
Sauvignon petit 111 Şt.	1.031 \pm 0.038	0.633 \pm 0.144	0.615 \pm 0.035
Chardonnay 15 Şt.	0.978 \pm 0.055	0.908 \pm 0.343	0.932 \pm 0.157
Tămăioasă Românească	0.953 \pm 0.060	0.527 \pm 0.224	0.557 \pm 0.092
Şarbă 2 Şt.	0.939 \pm 0.058	0.759 \pm 0.138	0.816 \pm 0.051
Ştefăneşti	0.926 \pm 0.075	0.696 \pm 0.254	0.752 \pm 0.033
Average	1.122	0.773	0.699
LSD 5%-absolute values	0.05	0.07	0.07
LSD 5%-relative values	4.5	9.1	10.0

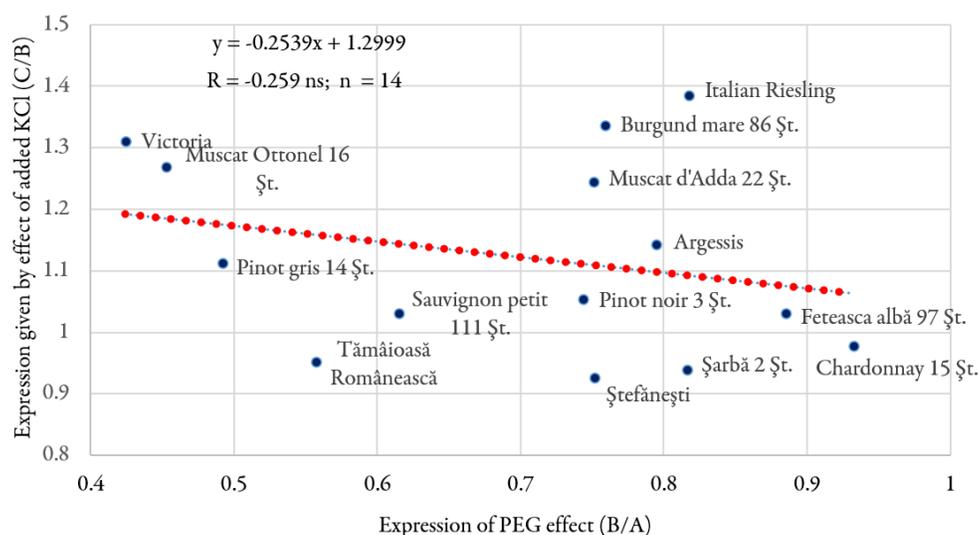


Figure 3. Relationship between effect of PEG (intrinsic OA expression) and addition of KCl (induced OA expression)

Blue line shows the global linear regression for all fourteen genotypes ($R = (-) 0.259$ (IS); $P < 0.05$)

The positive relationship established between ratios C/A and B/A was significant ($R = 0.821^{**}$) (Figure 4). The expression of overall osmotic adjustment (C/A) integrated that of intrinsic osmotic adjustment (B/A), explained by 50% of its variation (Figure 4).

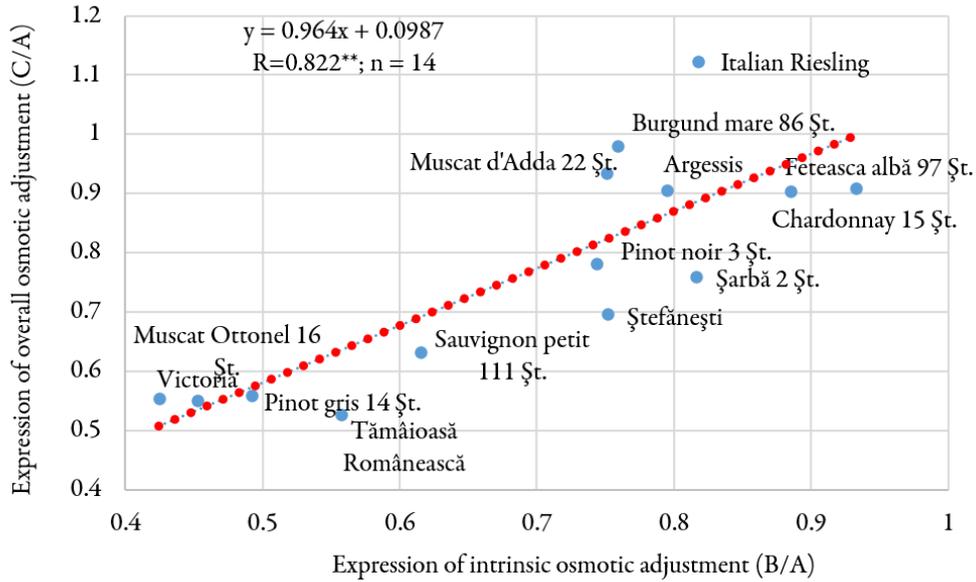


Figure 4. Relationship between intrinsic and overall OA expressions
Blue line shows the global linear regression for all fourteen genotypes ($R = (+) 0.822^{**}$; $P < 0.05$)

The positive relationship between overall and induced osmotic adjustment was not established (Figure 5), but several varieties such as ‘Italian Riesling’, ‘Burgund mare’ 86 Șt. and ‘Muscat d’Adda’ 22 Șt. had high mean values of ratio coefficients for both indicators (Figure 5).

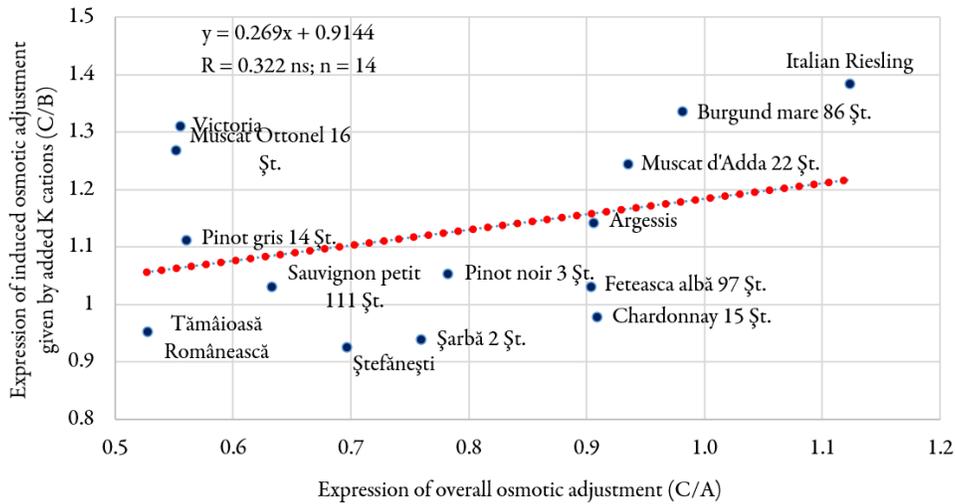


Figure 5. Relation between overall and induced OA expressions
Blue line shows the global linear regression for all fourteen genotypes ($R = (+) 0.322$ (IS); $P < 0.05$).

The overall analysis of correlations for all fourteen genotypes underlined several genotypes which are above regression line such as ‘Italian Riesling’, ‘Burgund mare’ 86 Șt., ‘Muscat d’Adda’ 22 Șt., ‘Muscat Ottonel’ 16 Șt. and ‘Victoria’, but ‘Muscat d’Adda’ 22 Șt. was closely kept in the linearity of the overall analysis interval (Figure 3; Figure 4; Figure 5).

Discussion

Responses differently expressed for intrinsic and induced osmotic adjustment in pollen grains suggested that grapevine genotypes might use different mechanisms that are activated at the time of water stress with different levels of solute accumulation. Rogiers *et al.* (2017) have been found that in grape varieties, aquaporins represent an important mechanism which control water flow through tissues and cells, but there are still important uncertainties that need to be resolved concerning those key processes that drive water accumulation in the cell. It has been suggested that the rapid increase in solute accumulation within the ripening grape berry mesocarp, including sugars, organic acids, K^+ and other cations such as Mg^{2+} drives osmotic water influx (Mpelasoka *et al.*, 2008; Rogiers *et al.*, 2017). After the cell turgescence is established, the cell extends wall and drives growth (Serpe and Matthew, 2000; Rogiers *et al.*, 2017).

Expression of intrinsic OA

The reason why two close responses were found for expression of intrinsic OA is because grapevine is generally a “drought-avoiding” specie (Chaves *et al.*, 1987; Schultz, 2003; Chaves *et al.*, 2010). Chaves *et al.* (2010) have been characterized the grapevine genotypes as “isohydric” (drought avoiders or “pessimistic”) and “anisohydric” with an “optimistic” response, which show a lower control over stomatal aperture under water stress (Schultz, 2003; Soar *et al.*, 2006; Chaves *et al.*, 2010). Studies modulated for ‘Chardonnay’s drought sensitivity characterized this variety as “anisohydric”. At the physiological level, stomata closure is one of the first response to water stress, in order to prevent the hydraulic failure (Charrier *et al.*, 2018; Cardone *et al.*, 2019). Intrinsic OA closely expressed by the mean values of 0.932 ± 0.157 in ‘Chardonnay’ 15 Şt., 0.885 ± 0.152 in ‘Fetească albă’ 97 Şt., 0.818 ± 0.090 in ‘Italian Riesling’ and 0.816 ± 0.051 in ‘Şarba’ 2 Şt. could indicate mechanisms involved in the ABA biosynthetic pathway (Chaves *et al.*, 2010), that are activated under drought conditions (Rossdeutsch *et al.*, 2016). Rossdeutsch *et al.* (2016) have been stated that an absolute relationship between high ABA production capacity and known drought tolerance in the field was not established, supporting that drought tolerance could be expressed through different mechanisms (Serra *et al.*, 2014).

Expression of induced OA

Addition of KCl to PEG solutions to which pollen grains were exposed, produced a change in the shape and size, either by restoring the initial size, or even increasing or reducing the pollen grain cytoplasm (David, 2012). ‘Italian Riesling’ increased pollen grains cytoplasm more than the other genotypes in PEG solutions with added KCl and had values >1 for both expressions of induced and overall OA. The results underlined the high K^+ accumulation capacity of ‘Italian Riesling’ as Bora *et al.* (2016) have been found it in their study.

Rogiers *et al.* (2017) have been stated that the pollen expressions of osmotic adjustment in grapevines genotypes occurred as a result of VvK 1.2 gene effect (inward rectifying shaker-like K^+ channel), on potassium transport, expressed in berry flesh, phloem tissues and perivascular cells of the vascular bundles. This gene is located on the chromosome 4 in the grapevine genome and it is expressed specifically than other genes involved in the osmotic adjustment expressions. Drought stress induced a two-to threefold increase in the VvK1.2 gene expression at post veraison, further substantiating that K^+ transport into berries is affected by drought stress (Cuéllar *et al.*, 2013). K^+ as an osmolyte, maintains pollen hydration and pollen tube growth to the ovary (Fan *et al.*, 2001; Rehman *et al.*, 2004). Therefore, the success of pollination and fertilization of the ovule, influenced by the capacity of K^+ accumulation in the cells, in all phenological stages (Rogiers *et al.*, 2017), determine the grape yield and quality.

K cation is very important for all stages of berry development (Hale, 1977; Hrazdina *et al.*, 1984; Rogiers *et al.*, 2006a; Martins *et al.*, 2012; Rogiers *et al.*, 2017). In the berry cells, K^+ is one of the varieties dependent and linked to ripening disorders late in ripening (Tilbrook and Tyerman, 2008; Fuentes *et al.*, 2010; Rogiers *et al.*, 2017)

Our study initiated to characterize the expressions of OA in the grapevine set found induced OA with implication of K^+ expressed differently in the pollen grains. Rogiers *et al.* (2017) have been explained the mechanisms in which K^+ could action differently in some grapevine genotypes at the cellular and sub-cellular levels. The lack of accumulation of K cations in the cell cytoplasm is generally influenced by backflow, phloem unloading, long distance phloem transport and retrieval phenomena. At the other hand, grapevine is a perennial plant which develops different adaptation pathways for each phenological stage under drought conditions. These might be a cause why some grapevine genotypes successfully accumulated K cations in all phenological stages than others and this difference in the capacity of K^+ accumulation could be used in the screening of grapevine tolerance.

Conclusions

The study found differences both in the measurements of projected cytoplasm area and expressions of osmotic adjustment in the grapevine pollen grains. Therefore, pollen grain test proposed by Morgan (1999) in wheat could be used as a quick screening method for establishing the level of drought sensitivity in grape plants.

The analysis initiated to characterize the expressions of OA in the grapevine set found induced OA with implication of K^+ expressed differently in the pollen grains. Because, the capacity of K^+ accumulation at cellular and sub-cellular levels is expressed in all phenological stages developed by grapevine and determine the yield and quality of grapes under drought conditions, drought sensitivity could be quantified through estimation of induced osmotic adjustment given by K^+ . Induced osmotic adjustment given by K^+ was expressed with the highest values in the pollen grains of several genotypes such as 'Italian Riesling', 'Burgund mare' 86 Şt., 'Muscat d'Adda' 22 Şt., 'Muscat Ottonel' 16 Şt., 'Pinot gris' 14 Şt. and 'Argessis' and with the lowest value in 'Tămâioasă Românească'.

Further research will be address to the induced osmotic adjustment expressed by K cations tested on a larger grapevine germplasm. These data in correlation with molecular information regarding VvK1.2 gene expression will be important for a complete drought tolerance characterization of the grapevine genotypes.

Authors Contributions

Conceptualization: MD; Data curation: DEV, IDT, AT, C-MC, MFB; Formal analysis: MD and AT; Investigation: MD and AT; Methodology: MD; Resources Software: National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti-Argeş; Writing-original draft: MD; Writing-review and editing: AT

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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